75. **Amended.** A fused gene encoding a humanized immunoglobulin heavy chain comprising:

a) a first nucleic acid molecule encoding a variable region derived from the murine 3D1 monoclonal antibody, comprising a framework region derived from the heavy chain of the human III2R antibody; and

b) a second nucleic acid sequence encoding at least a portion of a constant region of an immunoglobulin of human origin.

76. **Amended.** The humanized immunoglobulin of either of claims 1 or 10 which binds to human B7-2 with an affinity of about $1x10^{9}$ $M^{-1}$.

## REMARKS

Claims 1-12, 15, 21, 23-25, 27-28, 30-36, 38-40, 46, and 64-76 were under examination. Claims 7, 8, 9, 31, 33, 34, and 35 have been cancelled with out prejudice herein. Claims 1, 2, 10, 11, 25, 30, 46, 75, and 76 have been amended. Accordingly, claims 1-6, 10-12, 15, 21, 23-25, 27-28, 30, 32, 36, 38-40, 46, and 64-76 are currently under examination. Support for the amendments to the claims can be found in the specification and/or the claims as previously pending.

Applicant submits herewith a "Version with Markings to Show Changes Made," attached as Appendix A which indicates the specific amendments made to the claims and the specification.

No new matter has been added. Amendment and/or cancellation of the claims should in no way be construed as an acquiescence to any of the Examiner's rejections and was done solely to expedite prosecution. Applicants reserve the right to pursue the claims as originally filed in this or a separate application(s).

Since the claim amendments made herein are for purposes of clarifying claim language or incorporate subject matter previously claimed and examined in the present application into the currently pending claims, no additional search is required and no new

issues have been raised. Therefore, the claim amendments and cancellations made herein are permissible under 37 C.F.R. §1.116 as reducing the number of issues for appeal, and Applicants respectfully request that the present Amendment be entered.

The specification has been amended to incorporate pages 1639-1652 of The Journal of Experimental Medicine, 1991, Volume 174 as an "Appendix" after the last page of the specification. This amendment includes the entire disclosure of pages 1639-1652 of The Journal of Experimental Medicine, 1991, Volume 174. A copy of pages of pages 1639-1652 of The Journal of Experimental Medicine, 1991, Volume 174 is attached hereto. The amendatory material consists of the same material in which is referred to, e.g., at page 36 of the specification and incorporated by reference by the statement on page 54 of the instant specification. No new matter has been added.

The specification has also been amended to correct a typographical error; the term "I2R" has been replaced with the correct term "III2R." Support for this correction can be found at least at page 36 of the specification which indicates that human heavy chain framework sequences which were used to humanize the 3D1 antibody were from the human subgroup I (see e.g., page 36, lines 16-20). Page 36 of the specification also states that the heavy chain framework sequences were published by Manheimer-Lory, A. et al, J. Exp. Med. 174(6):1639-52(1991). Table I of the Manheimer-Lory reference teaches only 2 cell lines with heavy chain variable regions belonging to subtype I: the III-2R cell line and the R3.5H5G cell line. It is clear that the use of the term "I2R" rather than "III2R" throughout the specification was a typographical error. Accordingly, the term "I2R" has been replaced with "III2R" at each occurrence. No new matter has been added.

Withdrawal of Certain Rejections

Applicants gratefully acknowledge the withdrawal of the rejection of claims 1-4, 6-40, 46-49, and 50 as being anticipated by Freeman et al. (US Patent 6, 084,067).

Formal Drawings

The Examiner has objected to the drawings, which fail to comply with 37 CFR 1.142(b). Applicants will submit substitute formal drawings which comply with 37 CFR 1.142(b) upon indication from the Patent Office that the pending claims are in condition for allowance.

Rejection of Claims 1-12, 15, 21, 23-25, 27, 28, 30-36, 38-40, 46, and 64-76 Under 35 USC 112, First Paragraph

The rejection of Claims 1-12, 15, 21, 23-25, 27, 28, 30-36, 38-40, 46, and 64-76 under 35 USC 112, first paragraph has been maintained. More specifically the Examiner states:

> It is apparent that the "3D1" and "H2F", "I2R" antibodies and the CRL-12524 cell lines" are required to practice the claimed invention. As required elements, they must be known and readily available to the public or obtainable by a repeatable method set forth in the specification. If they are not so obtainable or available, the enablement requirements of 35 USC 112, first paragraph, may be satisfied by a deposit of the appropriate cell lines/hybridomas which produce these antibodies. ...
>
> Again, as pointed out previously, it has been noted that if the claimed and disclosed amino acid sequences or nucleic acid sequences set forth in the instant application encode the entire "3D1, "H2F" and "I2R" antibodies; then a deposit for said "antibodies" (cell lines/hybridomas) are not required. The sequence of the entire immunoglobulin satisfies the biological deposit of said immunoglobulin.

This rejection is respectfully traversed.

It is respectfully submitted that the generation of the antibodies of the present invention does not require the full length amino acid sequence of the murine 3D1, human H2F and human I2R antibodies. Rather the amino acid sequences of the light chain variable region of the human H2F antibody, the heavy chain variable region of the human III2R antibody, and the light chain and heavy chain variable regions of the 3D1 antibody each of which were known in the art or publicly available are sufficient to

practice the invention. The instant application teaches how to construct humanized 3D1 antibody using these sequences.

As taught in the specification, the amino acid sequence of the light chain variable region of the human H2F antibody was published (Manheimer-Lory et al., *J Exp. Med 174:* 1639-52 (1991)) and was known in the art. This reference was cited in the specification, e.g., at page 36 and was incorporated by reference at page 54 of the specification. The reference teaches the nucleotide sequence of the H2F light chain variable region (see Figure 3 of the reference). For convenience, a copy of this disclosure is enclosed and has been incorporated into the specification as an Appendix. Given the availability of this sequence information, it would not have been necessary for one of ordinary skill in the art to obtain the clone producing this antibody and sequence it themselves. Nucleic acid molecules encoding the light chain variable region could be readily synthesized using techniques well known in the art. Accordingly, this disclosure of the sequence of the H2F light chain variable region fulfills the requirements of 35 USC 112.

The specification also teaches that the amino acid sequence of the III2R heavy chain variable region of the human was published in Manheimer-Lory et al., (*J. Exp. Med. 174*: 1639-1652 (1991). Accordingly, this sequence was also known in the art. This reference was cited in the specification, e.g., at page 36 and was incorporated by reference at page 54 of the specification. The reference teaches the nucleotide sequence of the III2R heavy chain variable region (see Figure 7A of the reference). For convenience, a copy of this disclosure is enclosed and has been incorporated into the specification as an appendix. Given the availability of this sequence information, it would not have been necessary for one of ordinary skill in the art to obtain the clone producing this antibody and sequence it themselves. Nucleic acid molecules encoding the heavy chain variable region could be readily synthesized using techniques well known in the art. Accordingly, this disclosure of the sequence of the III2R heavy chain variable region fulfills the requirements of 35 USC 112.

In the interest of expediting prosecution and in no way conceding the validity of the Examiner's position, the specification has been amended to include the disclosure

Manheimer-Lory et al. 1991. J. Exp. Med. 174:1639. Furthermore, the specification has been amended in the interest of public policy considerations set forth in M.P.E.P. 608.01(p) in order to minimize the public's burden to search for and obtain copies of documents incorporated by reference.

Applicants have amended the specification pursuant to *In re Hawkins*, 486 F.2d 569, 179 USPQ 157 (CCPA 1973), in which it was decided that British applications which were incorporated by reference could later be inserted into the specification by amendment thereby ensuring a complete public disclosure. It was also found that this amendment did not constitute new matter. Applicants submit that, consistent with *In re Hawkins*, amendment of the instant specification to incorporate pages 1639-1652 of The Journal of Experimental Medicine, 1991, Volume 174 does not constitute new matter.

Applicants hereby state that the amendatory material consists of the same material in pages 1639-1652 of The Journal of Experimental Medicine, 1991, Volume 174 which is referred to, e.g., at page 36 of the specification and incorporated by reference by the statement on page 54 of the instant specification. No new matter has been added. The specification has been amended pursuant to 37 C.F.R. §1.121(b)(iii).

The sequences of the heavy chain variable region and the light chain variable region of the mouse 3D1 antibody are provided in the specification in Figure 1A (SEQ ID NOs: 1 and 2) and Figure 1B (SEQ ID NOs: 3 and 4), respectively. The hybridoma producing this antibody was deposited with the ATCC and accorded Accession Number HB 11686. If necessary, a Declaration for Deposit certifying that the deposit was made under conditions of the Budapest Treaty and stating the terms of the deposit can be provided.

Armed with the sequence of the heavy chain variable region of the III2R antibody, the sequence of the light chain variable region of the H2F antibody and the heavy and light chain variable regions of the murine 3D1 antibody, one of ordinary skill in the art could make the humanized antibodies of the instant invention. As taught in the specification, the framework regions of a compatible human antibody heavy chain and light chain variable region are assembled with the CDR's of the mouse monoclonal antibody. Changes made to the human framework region were determined by

comparison to the respective mouse variable region sequence. These changes are described in the Examples of the instant application.

Example 3 of the specification details methods by which the humanized antibodies of the invention can be assembled. Applicants teach methods for constructing plasmids comprising the light and heavy chain variable region genes by subcloning into the XbaI site of the pVk plasmid for expression of light chain and pVg4 or pVg2.M3 for expression of heavy chain. As is taught in that Example, the pVk is know in the art (see e.g., Co et al., J. Immunol. 148:1149 (1992)). The pVg4 vector was made by replacing the XbaI-BamHI fragment of pVg1 containing the g1 constant region gene (see e.g., Co et al., J. Immunol. 148:1149 (1992)) with an approximately 2000 bp fragment of the human g4 constant region gene (Ellison and Hood, Proc. Natl. Acad. Sci. USA 79:1984 (1982)). The pVg.M3 vector was described by Cole et al. J. Immunol. 159:3613 (1991). Because they are not involved in antigen recognition, the constant region amino acid sequences chosen are not critical. In fact, various constant region sequences can be chosen depending on the desired effector function of the humanized antibody. Using similar methods, a variety of constant region genes could be substituted for the specific regions taught.

It is Applicants' position that the sequences of the variable region genes are all that is required to make the claimed invention. Moreover, the constant regions used in making the instant humanized antibodies are known in the art and described in the specification.

With respect to the humanized 3D1 antibody, the amino acid sequence of the heavy chain and light chain variable regions is provided in the specification (Figure 2A (SEQ ID NOs: 5 and 6) and Figure 2B (SEQ ID NOs: 7 and 8, respectively). Applicants submit that the sequence information along with the teaching provided in the specification is sufficient to enable the claimed invention. Applicants also note that, while such a deposit is not believed to be necessary to meet the requirements of 35 USC §112, first paragraph, the hybridoma producing the humanized 3D1 antibody has been deposited with the A.T.C.C. and accorded Accession Number CRL-12524. A copy of the deposit receipt is attached as Appendix C. If necessary, a Declaration for Deposit

certifying that the deposit was made under conditions of the Budapest Treaty and stating the terms of the deposit can be provided.

Accordingly, Applicants respectfully request that the rejection under 35 USC § 112, first paragraph be reconsidered and withdrawn.

Rejection of Claims 1-12, 15, 21, 23-25, 27, 28, 30-36, 38-40, 46, and 64-76 under 35 USC 112, Second Paragraph

The rejection of Claims 1-12, 15, 21, 23-25, 27, 28, 30-36, 38-40, 46, and 64-76 under 35 USC 112, second paragraph has been maintained. The Examiner states:

> The use of "3D1" and "H2F", "I2R" antibodies as the sole means of identifying the claimed antibodies renders the claims indefinite because these "names' are merely laboratory designations which do not clearly define e the claimed products; since different laboratories may use the same laboratory designations to define completely distinct cell lines or hybridomas.

This rejection is traversed and met in part. Applicants submit that the term "3D1" is clearly defined in the specification and is known in the art. Given Applicants' teachings with respect to the sequence of the heavy and light chain variable regions of the 3D1 antibody (see, e.g., SEQ ID Nos 1-4), it is Applicants' position that the term as used is definite. It is further Applicants' position that the terms "H2F" and "III2R" are also known in the art. In addition, Applicants have amended the specification to include the H2F and III2R variable region sequences. Accordingly, it is Applicants' position that the terms as used are definite. In light of the foregoing, Applicants respectfully request that this rejection be reconsidered and withdrawn.

The Examiner further states:

> Claims 24 and 28 are indefinite in the recitation of "stringent conditions" because the metes and bounds of such conditions are ambiguous and unclear and in turn the metes and bounds of the claimed "nucleic acids" are not defined.

This rejection is respectfully traversed. Applicants submit that the term "stringent hybridization conditions" was an art recognized term at the time of the invention. For instance, "stringent hybridization conditions" are described in Ausubel, F.M. *et al.*, eds.

*Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons Inc., (1993). Specifically, the reference teaches that stringent hybridization conditions are at least as stringent as 48% formamide and 1X SSC at 42°C or 0.5M NaHPO4 at 65°C with wash conditions at least as stringent as 0.2X SSC, 0.1% SDS at 65°C or 40mM NaHPO4 at 65°C. This reference is cited in the second full paragraph of page 27 of the specification and incorporated by reference at page 54 of the specification. Therefore, one of ordinary skill in the art would have known what Applicants regarded as the invention with respect to this term. Accordingly, Applicants submit that the term "stringent hybridization conditions" is definite and respectfully request reconsideration and withdrawal of the rejection of claims 24 and 28 under 35 USC §112, second paragraph.

Rejection of Claims 1-12, 15, 21, 23-25, 27, 28, 30-36, 38-40, 46, and 64-76 under 35 USC 103(a)

Claims 1-12, 15, 21, 23-25, 27, 28, 30-36, 38-40, 46, and 64-76 have been rejected under 35 USC 103(a) "as obvious over Freeman et al. (U.S. Patent No. 6,084,067) in view of art known gene cloning and expression strategies for deriving recombinant antibodies and fragments thereof." More specifically the Examiner states:

> [I]t would have been [have been] a matter of routine experimentation well within the ordinary skill level of art to generate chimeric, humanized or recombinant HF2.3D1-B7-2-specific antibodies, nucleic acids encoding said antibodies, vectors, host cells, methods of making and compositions thereof; given the HF2.3D1 antibody and hybridoma and its associated properties known in the prior art.

The Examiner further states:

> it was obvious to one of ordinary skill in the art at the time the invention was made to humanize various antibodies, including "F2.3D1" B7-2-specific antibody, particularly in view of its specificity and functional properties known at the time the invention was made.

> Given the availability of the HF2.3D/3D1 antibody and hybridoma together with general immunoglobulin gene cloning and expression strategies, it would have been [have been] a matter of routine experimentation well within the ordinary skill level of art to generate chimeric or humanized HF2.3D1/3D1 antibody B7-2 specific antibodies, nucleic acids encoding said antibodies, vectors, host cells, methods of making and compositions thereof. Given the highly conserved nature of immunoglobulin gene organization and structure and the availability of probes and PCR primers for immunoglobulin gene cloning, one of ordinary skill in the art could have isolated the functionally rearranged heavy and light chain variable regions from the HF2.3D1 hybridoma cell line and determined their sequences with a complete expectation of success.

This rejection is respectfully traversed.

To establish a *prima facie* case of obviousness for the claimed invention, there must have been some suggestion or motivation, either in the cited references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings in the manner proposed by the Examiner. Second, there must have been a reasonable expectation of success at the time the invention was made. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. See M.P.E.P. 2143. The prior art must suggest "to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process" and "[b]oth the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure." *In re Dow Chemical Co.* 837 F.2d 469. 473, 5 U.S.P.Q.2d 1529, 1531 (Fed.Cir. 1988).

The pending claims are directed to humanized immunoglobulins having binding specificity for B7-2, and ***comprising at least one complementarity determining region derived from the murine 3D1 antibody and, a human light chain framework region derived from the light chain of the human H2F antibody, and a human heavy chain framework region derived from the heavy chain of the human III2R antibody***.

The pending claims are further directed to humanized immunoglobulins having binding specificity for B7-2 which humanized immunoglobulin is ***derived from the cell line deposited with the ATCC®, Accession No. CRL-12524***.

The pending claims are still further directed to humanized immunoglobulin light chains having binding specificity for B7-2 ***comprising CDR1, CDR2 and CDR3 of the light chain of the murine 3D1 antibody, and a human light chain framework region derived from the light chain of the human H2F antibody***.

The pending claims are further directed to isolated nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of: (a) ***SEQ ID NO:7***, (b) a nucleotide sequence encoding the amino acid sequence of ***SEQ ID NO:8***, (c) the nucleic acid sequence of a nucleic acid molecule which ***hybridizes to the nucleic acid molecule comprising a nucleotide sequence according to a) or b) under stringent hybridization conditions***, and d) a nucleotide sequence which is ***the complement of the nucleotide sequence according to a) or b)***.

The pending claims are also directed to humanized immunoglobulin heavy chains specific for B7-2 comprising ***CDR1, CDR2 and CDR3 of the heavy chain of the murine 3D1 antibody, and a human heavy chain framework region derived from the heavy chain of the human III2R antibody.***

The pending claims are also directed to isolated nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of: a)***SEQ ID NO: 5,*** b) a nucleotide sequence encoding the amino acid sequence of ***SEQ ID NO:6***, c) the nucleotide sequence of a nucleic acid molecule which ***hybridizes to the nucleic acid molecule comprising a nucleotide sequence according to a) or b) under stringent hybridization conditions***, and d) a nucleotide sequence which is ***the complement of the nucleotide sequence according to a) or b)***.

The pending claims are yet further directed to a fused gene encoding a humanized immunoglobulin light chain ***comprising a first nucleic acid molecule encoding a variable region derived from the murine 3D1 monoclonal antibody, comprising a framework region derived from the light chain of the human H2F antibody***; and a second nucleic acid sequence encoding at least a portion of a constant region of an immunoglobulin of human origin.

The pending claims are yet further directed to a fused gene encoding a humanized immunoglobulin heavy chain comprising: ***a first nucleic acid molecule encoding a variable region derived from the murine 3D1 monoclonal antibody, comprising a framework region derived from the heavy chain of the human III2R antibody***; and a second nucleic acid sequence encoding at least a portion of a constant region of an immunoglobulin of human origin.

It is Applicants' position that the general guidelines present in the prior art with respect to making humanized antibodies merely provide a foundation for further research. Applicants submit that it requires an inventive contribution to the art to successfully make and select humanized antibodies that maintain their ability to bind antigen. In addition, Applicants submit that, at the time the invention was made, there was no teaching or suggestion in the art to use either the human III2R or H2F antibody sequences for humanization of the murine 3D1 antibody, let alone to use both III2R heavy chain variable region sequences and H2F light chain variable region sequences to humanize the 3D1 antibody.

First, the prior art teaches that use of these general guidelines does not always result in a humanized antibody with antigen binding properties comparable to those of the starting murine antibody. For example, the specification teaches that the humanized antibodies of the invention were made using the general teachings of Queen et al. PNAS 86:10029 (1989) (see, e.g., page 36 of the specification at line 5, copy attached as

Appendix D) At page 10032 the reference teaches that the CDR grafted antibodies taught therein have approximately one third the binding activity of the starting murine antibodies. At page 16 of the specification, Applicants also teach that the teachings of Co et al., Proc. Natl. Acad. Sci. USA 88:2869 (1991) (Attached as Appendix E) can be followed to produce humanized antibodies. At page 2869, Co et al. teach that "generation of other fully humanized antibodies has proved unexpectedly difficult, because significant loss of binding affinity generally resulted from simple grafting of hypervariable regions, probably due to distortion of the complementarity-determining region (CDR) conformation by the human framework." Other references also teach that CDR replacement into human framework regions can lead to significant loss of binding affinity to the antigen (see, e.g., Tempest et al., Biotechnology 9:266 (1992) or Shalaby et al. J. Exp. Med. 17:217 (1992), cited in the specification). Thus, an inventive contribution must be made in order to successfully humanize each individual antibody.

Applicants' inventive contribution included the identification of human framework regions appropriate for use with the mouse 3D1 antibody, and the identification of amino acid residues in the human framework regions to be replaced with amino acids from the original 3D1 antibody. All of the pending claims require the presence of a framework region derived from at least one of these antibodies. Absent any teaching or suggestion to use the specific human acceptor variable region framework regions of the III2R or H2F antibodies to humanize the 3D1 antibody as presently claimed, there would have been no motivation to use these human framework regions to humanize the 3D1 antibody. The existence of general principles for producing humanized antibodies is not sufficient to render the claimed invention obvious. The patentability of a composition claim is to be determined from the structure of the composition itself and not the existence of a general method for making such a composition. "The existence of a general method . . . is essentially irrelevant to the

question whether the specific molecules themselves would have been obvious." *In re Duel*, 34 USPQ2e 1210, 1214 (Fed. Cir. 1995)

Claims 1 and 15 and the claims that depend therefrom of the application require that both III2R and H2F sequences be present in the claimed antibody. In addition to there being no motivation present in the art to select framework sequences from these two human antibodies, selected out of the entire class of human antibodies, at the time the invention was made, there would have been no reasonable expectation of success in making the claimed humanized antibodies. The art taught that it was not desirable to use heavy and light chain framework regions from different antibodies. For example, Co et al. taught that "[n]ormally the heavy chain and light chain from the same human antibody are chosen so as to reduce the possibility of incompatibility in the assembly of the two chains" (supra at page 2871). Accordingly, at the time the invention was made, there was no reasonable expectation of success that a humanized antibody comprising framework sequences from two different human antibodies would assemble properly.

Claims 23, 24, 27, and 28 and the claims that depend therefrom require that the humanized antibody comprise a specific amino acid sequence or be encoded by a specific nucleotide sequence. Claim 15 requires that the humanized antibody be produced by the cell line which produces the humanized 3D1 antibody. Thus, each of these claims requires the specific sequences of the light and/or heavy chain of humanized 3D1 antibody taught in the application. The humanized 3D1 antibody comprises positions in the human acceptor variable region framework sequence which were substituted with corresponding amino acids from the donor mouse antibody. At amino acid positions 3 and 22 the light chain of the humanized 3D1 antibody amino acids from the murine 3D1 antibody were substituted for the original human framework amino acids. At amino acid positions 27, 30, 48, 67, 68, 70, 72, and 113 the heavy chain of the humanized 3D1 antibody amino acids from the murine 3D1 antibody were substituted for the original

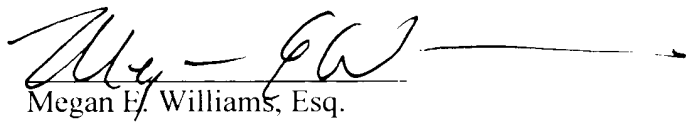
human framework amino acids. The amino acid substitutions required an inventive contribution by Applicants. The cited art is devoid of any teaching or suggestion to change these claimed residues. Thus the cited art fails to teach or suggest all the limitations of the claims as required by M.P.E.P. 2143.

## SUMMARY

In view of the foregoing remarks, reconsideration of the rejections and allowance of all pending claims is respectfully requested.

If a telephone conversation with Applicants' attorney would expedite the prosecution of the above-identified application, the examiner is urged to call Applicants' attorney at (617) 227-7400.

Respectfully submitted,



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Dated: December 20, 2001

**Version With Markings to Show Changes Made**

The paragraph at page 2, beginning at line 20 has been replaced with:

The invention also embodies a humanized immunoglobulin having a binding specificity for B7-2 comprising a heavy chain and/or a light chain. The light chain comprises a CDR (e.g., CDR1, CDR2 and CDR3) derived from an antibody of nonhuman origin which binds B7-2 and a FR derived from a light chain of human origin (e.g., H2F antibody). The heavy chain comprises a CDR (e.g.,CDR1, CDR2 and CDR3) derived from an antibody of nonhuman origin which binds B7-2 and a FR region derived from a heavy chain of human origin (e.g., the human [I2R] III2R antibody). The immunoglobulin can further comprise CDR1, CDR2 and CDR3 for the light or heavy chain having the amino acid sequence set forth herein or an amino acid

Please replace the paragraph at page 3, beginning at line 18 with:

Another embodiment of the invention is a humanized immunoglobulin heavy chain that is specific for B7-2 and comprises CDR1, CDR2 and/or CDR3 of the heavy chain of the 3D1 antibody, and a human heavy chain FR (e.g., [I2R] III2R antibody). The invention pertains to a humanized immunoglobulin heavy chain that comprises a variable region shown in Figure 2A ( SEQ ID NO: 6). The invention also pertains to an isolated nucleic acid sequence that encodes a humanized variable heavy chain specific for B7-2 that comprises a nucleic acid, such as the sequence shown in Figure 2A (SEQ ID NO: 5), a nucleic acid that encodes the amino acid sequence shown in Figure 2A (SEQ ID NO: 6), a nucleic acid which hybridizes thereto under stringent hybridization conditions, and a nucleic acid which is the complement thereof.

The paragraph at page 36, beginning at line 4 has been replaced with:

To retain the binding affinity of the mouse antibody in the humanized antibody, the general procedures of Queen *et al.* were followed (Queen *et al. Proc. Natl. Acad. Sci. USA 86*: 10029 (1989), U.S. Patent Nos. 5,585,089 and 5,693,762, the teachings of which are incorporated herein in their entirety). The choice of framework residues can be critical in retaining high binding affinity. In principle, a framework sequence from any

human antibody can serve as the template for CDR grafting; however, it has been demonstrated that straight CDR replacement into such a framework can lead to significant loss of binding affinity to the antigen (Tempest *et al.*, *Biotechnology* 9: 266 (1992); Shalaby *et al.*, *J. Exp. Med. 17*: 217 (1992)). The more homologous a human antibody is to the original murine antibody, the less likely the human framework will introduce distortions into the mouse CDRs that could reduce affinity. Based on a sequence homology, [I2R] III2R was selected to provide the framework for the humanized 3D1 heavy chain and H2F for the humanized 3D1 light chain variable region. Manheimer-Lory, A. *et al.*, *J. Exp. Med.* 174(6):1639-52 (1991). Other highly homologous human antibody chains would also be suitable to provide the humanized antibody framework, especially kappa light chains from human subgroup 4 and heavy chains from human subgroup 1 as defined by Kabat.

The paragraph at page 36, beginning at line 21 (and continuing to line 3 of page 37) has been replaced with:

Normally the heavy chain and light chain from the same human antibody are chosen to provide the framework sequences, so as to reduce the possibility of incompatibility in the assembling of the two chains. The [I2R] III2R antibody shows a high homology to the 3D1 heavy and light chains and thus, was chosen to provide the framework for the initial humanized antibody model. The 3D1 light chain variable region, however, shows a significantly higher homology to the H2F framework compared to any others, including [I2R] III2R. Therefore, H2F was chosen instead to provide the framework for the humanized 3D1 light chain variable region, while [I2R] III2R was selected to provide the framework for the heavy chain variable region.

The paragraph at page 37, beginning at line 4 has been replaced with:

The computer programs ABMOD and ENCODE (Levitt *et al.*, *J. Mol. Biol. 168:* 595 (1983)) were used to construct a molecular model of the 3D1 variable domain, which was used to locate the amino acids in the 3D1 framework that are close

enough to the CDRs to potentially interact with them. To design the humanized 3D1 heavy and light chain variable regions, the CDRs from the mouse 3D1 heavy chain were grafted into the framework regions of the human [I2R] III2R heavy chain and the CDRs from the mouse 3D1 light chain grafted into the framework regions of the human H2F light chain. At framework positions where the computer model suggested significant contact with the CDRs, the amino acids from the mouse antibody were substituted for the original human framework amino acids. For humanized 3D1, this was done at residues 27, 30, 48, 67, 68, 70 and 72 of the heavy chain and at residue 22 of the light chain. Furthermore, framework residues that occurred only rarely at their positions in the database of human antibodies were replaced by a human consensus amino acid at those positions. For humanized 3D1 this was done at residue 113 of the heavy chain and at residue 3 of the light chain.

The paragraph at page 39, beginning at line12 has been replaced with:

Likewise, many of the framework residues not in contact with the CDRs in the humanized 3D1 heavy and light chains can accommodate substitutions of amino acids from the corresponding positions of [I2R] III2R and H2F frameworks, from other human antibodies, from the mouse 3D1 antibody, or from other mouse antibodies, without significant loss of the affinity or non-immunogenicity of the humanized antibody. Table 2 lists a number of additional positions in the framework where alternative amino acids may be suitable.

1. **Amended.** A humanized immunoglobulin having binding specificity for B7-2, said immunoglobulin comprising [an antigen binding] at least one complementarity determining region [of non-human origin] derived from the murine 3D1 antibody and [at least a portion of an immunoglobulin of human origin wherein the antigen binding] , a human light chain framework region derived from the light chain of the human H2F

antibody, and a human heavy chain framework region derived from the heavy chain of the human III2R [region comprises a heavy chain derived from the I2R] antibody [or a light chain derived from the H2F antibody].

2. **Amended.** The humanized immunoglobulin of Claim 1, wherein the [portion of an immunoglobulin of human origin is derived from] antibody comprises a human constant region.

7. **Cancel.** The humanized immunoglobulin of Claim 1, wherein the antigen binding region is of rodent origin.

8. **Cancel.** The humanized immunoglobulin of Claim 1, wherein the antigen binding region comprises a complementarity determining region of rodent origin, and the portion of an immunoglobulin of human origin is derived from a human framework region.

9. **Cancel.** The humanized immunoglobulin of Claim 8, wherein the complementarity determining region is derived from the 3D1 monoclonal antibody.

10. **Amended.** The humanized immunoglobulin of claim [9] 1, further comprising a constant region of human origin, wherein the heavy chain comprises a variable region of SEQ ID NO:6 and the light chain comprises a variable region of SEQ ID NO:8.

11. **Amended.** The humanized immunoglobulin of [any one of Claims 1 or 10] claim 1, wherein said immunoglobulin can compete with the murine 3D1 antibody for binding to B7-2.

25. **Amended.** A humanized immunoglobulin heavy chain specific for B7-2 comprising CDR1, CDR2 and CDR3 of the heavy chain of the murine 3D1 antibody, and a human heavy chain framework region derived from the heavy chain of the human [I2R] III2R antibody.

30. **Amended.** An expression vector comprising a fused gene encoding a humanized immunoglobulin [light chain, said gene comprising a nucleotide sequence encoding a CDR derived from a light chain of a nonhuman antibody having binding specificity for

B7-2 and a framework region derived from the light chain of the human H2F antibody] of claim 1.

31. **Cancel.** The expression vector of Claim 30, wherein the nonhuman antibody is the murine 3D1 antibody.

33. **Cancel.** An expression vector comprising a fused gene encoding a humanized immunoglobulin heavy chain, said gene comprising a nucleotide sequence encoding a CDR derived from a heavy chain of a nonhuman antibody having binding specificity for B7-2 and a framework region derived from the heavy chain of the human I2R antibody.

34. **Cancel.** The expression vector of Claim 33, wherein the nonhuman antibody is the murine 3D1 antibody.

35. **Cancel.** A host cell comprising the expression vector of Claim 33.

46. **Amended.** A pharmaceutical composition comprising the antibody of [any one of Claims 1 or 10,] claim 1 and a pharmaceutically acceptable carrier.

75. **Amended.** A fused gene encoding a humanized immunoglobulin heavy chain comprising:

a) a first nucleic acid molecule encoding [an antigen binding] a variable region derived from the murine 3D1 monoclonal antibody, comprising a framework region derived from the heavy chain of the human [I2R] III2R antibody; and

b) a second nucleic acid sequence encoding at least a portion of a constant region of an immunoglobulin of human origin.

76. **Amended.** The humanized immunoglobulin of [any one] either of claims 1 or 10 which binds to human B7-2 with an affinity of about $1x10^{9}$ $M^{-1}$.